

# Stomatal Sensitivity to Carbon Dioxide and Humidity

## A COMPARISON OF TWO C<sub>3</sub> AND TWO C<sub>4</sub> GRASS SPECIES

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### ABSTRACT

The sensitivity of stomatal conductance to changes of CO<sub>2</sub> concentration and leaf-air vapor pressure difference (VPD) was compared between two C<sub>3</sub> and two C<sub>4</sub> grass species. There was no evidence that stomata of the C<sub>4</sub> species were more sensitive to CO<sub>2</sub> than stomata of the C<sub>3</sub> species. The sensitivity of stomatal conductance to CO<sub>2</sub> change was linearly proportional to the magnitude of stomatal conductance, as determined by the VPD, the same slope fitting the data for all four species. Similarly, the sensitivity of stomatal conductance to VPD was linearly proportional to the magnitude of stomatal conductance. At small VPD, the ratio of intercellular to ambient CO<sub>2</sub> concentration, C<sub>i</sub>/C<sub>a</sub>, was similar in all species (0.8–0.9) but declined with increasing VPD, so that, at large VPD, C<sub>i</sub>/C<sub>a</sub> was 0.7 and 0.5 (approximately) in C<sub>3</sub> and C<sub>4</sub> species, respectively. Transpiration efficiency (net CO<sub>2</sub> assimilation rate/transpiration rate) was larger in the C<sub>4</sub> species than in the C<sub>3</sub> species at current atmospheric CO<sub>2</sub> concentrations, but the relative increase due to high CO<sub>2</sub> was larger in the C<sub>3</sub> than in the C<sub>4</sub> species.

Atmospheric carbon dioxide concentration is rising as a result of the burning of fossil fuels (e.g. 8). This may increase the water use efficiency of vegetation through stomatal closure and the stimulation of net photosynthesis. However, plant species may not all respond to the same degree. It is a widely held view that stomata of C<sub>4</sub> species are more CO<sub>2</sub>-sensitive than are those of C<sub>3</sub> species (e.g. 1, 15, 19, 21) but few data support this notion unequivocally. Early workers compared transpiration rates while the ambient CO<sub>2</sub> concentration was changed in large steps and the humidity around the leaf varied with the transpiration rate, possibly affecting stomatal aperture directly (e.g. 1, 21). The most definitive comparison was that between the C<sub>4</sub> species *Zea mays* and *Amaranthus powelli* and the C<sub>3</sub> species *Xanthium strumarium* and *Gossypium hirsutum* (3). In that study, the two C<sub>4</sub> species exhibited higher sensitivity of leaf conductance to intercellular CO<sub>2</sub> concentration than did the C<sub>3</sub> species, unless ABA was supplied when, surprisingly, the ranking was reversed.

In making such comparisons, it is necessary to distinguish between the C<sub>a</sub><sup>1</sup> and the C<sub>i</sub>, since C<sub>i</sub> rather than C<sub>a</sub> is usually assumed to affect the guard cells. A difference in stomatal sensitivity to C<sub>a</sub> might not reflect a difference in the guard cell response to CO<sub>2</sub> but rather a difference in the ratio of C<sub>i</sub> to C<sub>a</sub> that is partly determined by net CO<sub>2</sub> assimilation rate (A) (2, 12, 29). Though

C<sub>i</sub>/C<sub>a</sub> is reported to be larger in C<sub>3</sub> species than in C<sub>4</sub> species (3, 14, 24, 29), it has been shown to be independent of C<sub>a</sub> and irradiance (17, 30), leaf age, and nutrient status (31). Therefore, C<sub>i</sub>/C<sub>a</sub> has been used in models of crop photosynthesis and water use efficiency (e.g. 27). However, large decreases in C<sub>i</sub>/C<sub>a</sub> have been observed as leaf temperature increases (11) and C<sub>i</sub>/C<sub>a</sub> must alter when stomatal conductance is affected directly by VPD independently of bulk leaf water status (e.g. 2, 6, 26). In this study, we compared the sensitivity of stomatal conductance to C<sub>a</sub> and C<sub>i</sub> between two C<sub>3</sub> and two C<sub>4</sub> grasses and examined the effect of humidity (VPD) on the sensitivity of stomatal conductance to CO<sub>2</sub>. Further, we examined the responses of C<sub>i</sub>/C<sub>a</sub> and A/E to C<sub>a</sub> and VPD in both C<sub>3</sub> and C<sub>4</sub> species.

### MATERIALS AND METHODS

**Plant Material.** Plants of maize (*Zea mays* L. cv XL399 [C<sub>4</sub>]), paspalum (*Paspalum plicatulum* Michx. cv Rudd's Bay [C<sub>4</sub>]), rice (*Oryza sativa* L. cv IR8 [C<sub>3</sub>]), and phalaris (*Phalaris aquatica* L. cv Sirosa [C<sub>3</sub>]) were grown in an artificially illuminated growth cabinet (LBH-type cabinet, Canberra phytotron [18]). Pregerminated seeds were planted into 3 dm<sup>3</sup> of soil (a river loam, sand, vermiculite, fertilizer mixture) in 50-cm sections of 9-cm diameter drainage pipe lined with sealed polyethylene sleeving. Water was added to the soil surface as required to maintain a water table at a constant 10 cm from the base of the pot. Photosynthetic photon flux density at plant height was 630 μmol (quanta) m<sup>-2</sup> s<sup>-1</sup> for a 16-h day, with a 25/20°C day/night cycle at constant 70% RH, corresponding to a VPD cycle of 0.95/0.70 kPa. At the time of leaf gas exchange measurements, the maize plants were 3 weeks old and rice plants were 11 weeks old. Paspalum and phalaris, both forage species, had been subject to several cycles of defoliation and regrowth as part of another experiment, but leaves used were 2 weeks old and fully expanded.

**Leaf Gas Exchange Measurements.** Measurements were made on single, attached leaves of two plants per species in another controlled environment cabinet. Eight cm<sup>2</sup> of leaf lamina were clamped into an aluminum, brass, and glass leaf chamber. Parts of the plant outside the leaf chamber were shaded and covered in a plastic bag to minimize evaporation losses. The light sources were a 1,000-w metal-halide lamp (HPLR, Philips, Holland) and a bank of fluorescent tubes. The photosynthetic photon flux density on the upper leaf surface was 670 ± 15 μmol (quanta) m<sup>-2</sup> s<sup>-1</sup>, measured with a photocell calibrated *in situ* against a quantum sensor (model LI-190; Lambda Instruments Co. Inc.). The leaf temperature used to calculate the saturated vapor pressure in the intercellular spaces was the mean reading from three copper-constantan thermocouple junctions pressed to the abaxial surface of the leaf. Leaf temperature was kept constant at 25.3 ± 0.15°C irrespective of transpiration rate. Boundary layer conductance for water vapor transfer was estimated from wet filter paper to be 2.1 mol m<sup>-2</sup> s<sup>-1</sup>. The vapor pressures of airstreams leaving and

<sup>1</sup> Abbreviations: C<sub>a</sub>, ambient CO<sub>2</sub> concentration; C<sub>i</sub>, intercellular CO<sub>2</sub> concentration; A, net CO<sub>2</sub> assimilation rate; VPD, leaf-air vapor pressure difference(s); A/E, transpiration efficiency; g<sub>s</sub>, stomatal conductance; Ψ, leaf water potential.

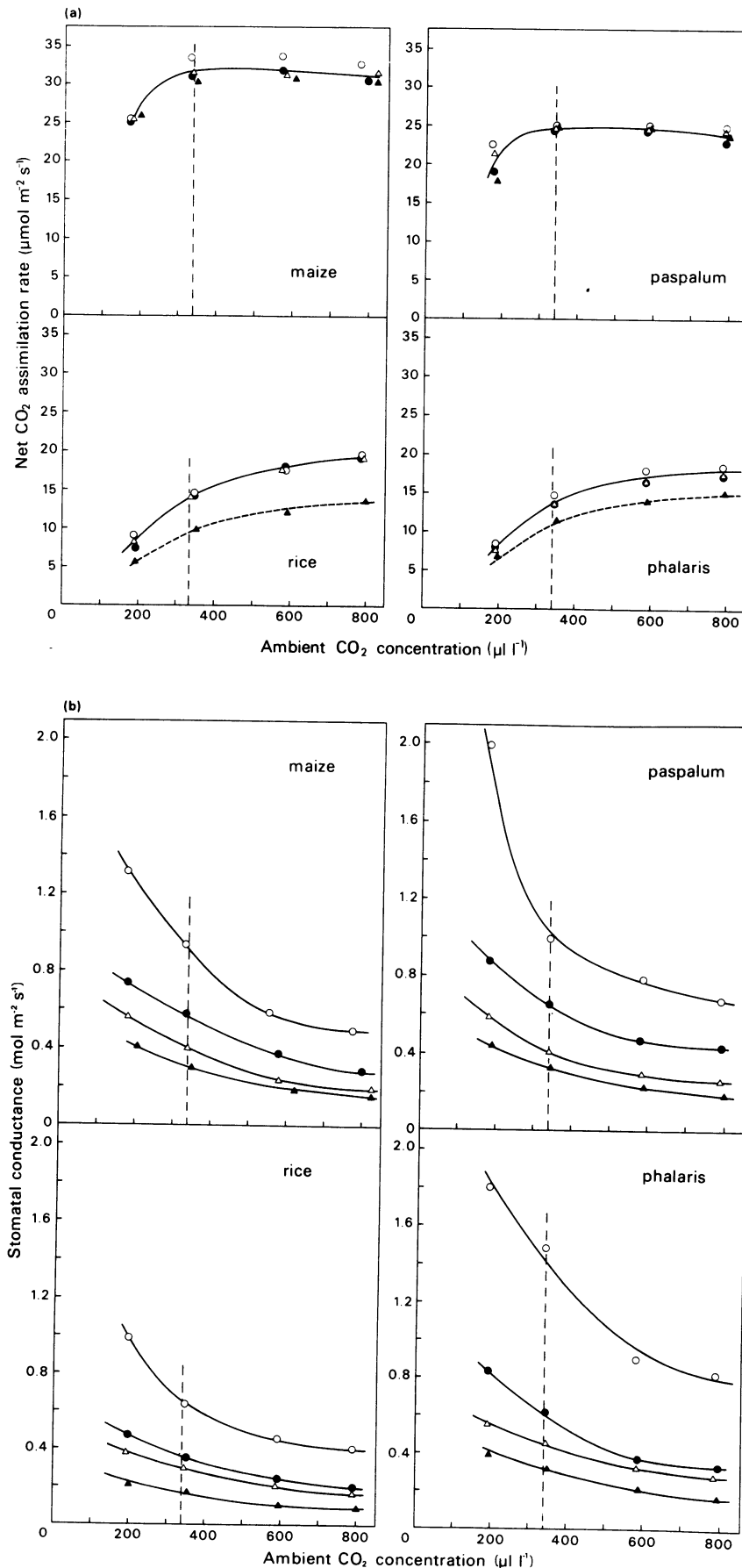


FIG. 1. Response of (a) net CO<sub>2</sub> assimilation rate and (b) stomatal conductance to ambient CO<sub>2</sub> concentration at four levels of VPD in the C<sub>4</sub> species maize and paspalum and the C<sub>3</sub> species rice and phalaris. Leaf-air vapor pressure differences are 0.5 pKa (○), 0.9 pKa (●), 1.4 pKa (△), and 2.0 pKa (▲). Vertical dashed lines indicate values of A and g<sub>s</sub> at C<sub>a</sub> = 340 μl l<sup>-1</sup>. The curves of g<sub>s</sub> versus C<sub>a</sub> are fitted quadratic functions.

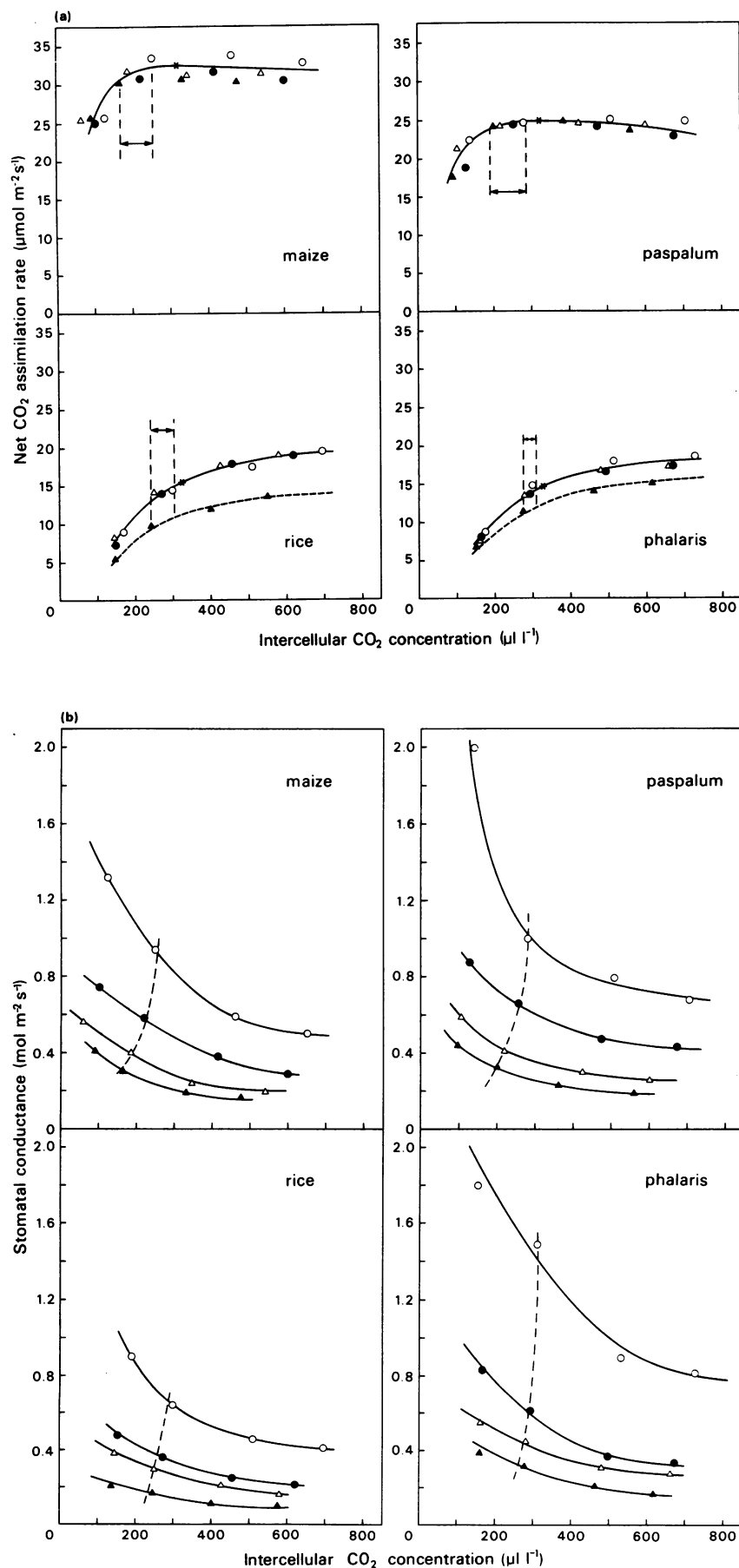


FIG. 2. Response of (a) net assimilation rate and (b) stomatal conductance to intercellular CO<sub>2</sub> concentration at four levels of VPD; data replotted from Figure 1. Dashed lines join values of  $g_s$  versus  $C_i$  for  $C_a = 340 \mu\text{l l}^{-1}$  as VPD varied. Horizontal arrows denote the range of  $A$  versus  $C_i$  at  $C_a = 340 \mu\text{l l}^{-1}$  as VPD varied. (\*), calculated values of  $A$  and  $C_i$  at infinite stomatal conductance and  $C_a = 340 \mu\text{l l}^{-1}$  (see "Discussion"). The curves of  $g_s$  versus  $C_i$  are fitted quadratic functions.

bypassing the chamber were measured with two thin-film capacitance humidity sensors (model 6061HM; Vaisala OY, Helsinki, Finland) kept at a constant temperature of 35.2°C, and also with a dewpoint hygrometer (model 880; EG & G, MA). The vapor pressure of the air around the leaf was kept constant at any desired value ( $\pm 0.02$  kPa) by adjusting the mixture of wet and dry air streams entering the chamber. Accurately known CO<sub>2</sub> concentrations were supplied by three gas mixing pumps in cascade (models SA18/3, M300/aF, G27/3F; Wösthoff oHG, D463 Bochum, Germany). CO<sub>2</sub> uptake in the chamber was measured by a differential IR gas analyzer (model 865; Beckman Instruments) fitted with interference filters to eliminate the cross-sensitivity to water vapor.

Calculations of transpiration rate (E), net CO<sub>2</sub> assimilation rate (A), stomatal conductance to water vapor (g<sub>s</sub>), C<sub>a</sub>, and C<sub>i</sub> included corrections due to atmospheric pressure (mean = 97 kPa), due to the counterflow of H<sub>2</sub>O on C<sub>i</sub>, and due to the dilution of C<sub>a</sub> by water vapor (as detailed in Ref. 28). All measurements are expressed on a projected leaf area basis.

**Procedure.** The response of assimilation rate, transpiration rate, and stomatal conductance to four ambient CO<sub>2</sub> concentrations were measured at four values of VPD over 2 d for each of two leaves per species. The CO<sub>2</sub> concentration supplied by the pumps was changed in the sequence: 360, 600, 800, and 200  $\mu\text{l l}^{-1}$ , with over 1 h equilibration at each concentration; the CO<sub>2</sub> concentration in the chamber, C<sub>a</sub>, was up to 25  $\mu\text{l l}^{-1}$  lower. On the 1st d, the CO<sub>2</sub>-response curves were measured at about 0.5 and 0.9 kPa VPD, and on the second at about 1.4 and 2.0 kPa VPD.

## RESULTS

**The Primary Data.** The response of A and g<sub>s</sub> to C<sub>a</sub> is shown in Figure 1. As expected, net assimilation rate saturated at a lower C<sub>a</sub> in the two C<sub>4</sub> species maize and paspalum than in the two C<sub>3</sub> species rice and phalaris. Net assimilation rate in all four species was largely unaffected by the VPD changing from 0.5 to 1.4 kPa, but the large VPD of 2 kPa (twice that prevailing during growth) caused a lower net assimilation rate in the two C<sub>3</sub> species, possibly as a result of the onset of water stress at high evaporation rates

Table I. Sensitivity of Stomatal Conductance to Changes in C<sub>i</sub> and C<sub>a</sub>

The sensitivity to C<sub>a</sub>, dg<sub>s</sub>/dC<sub>a</sub>, is the slope of g<sub>s</sub> versus C<sub>a</sub>, derived from the fitted quadratic function, at C<sub>a</sub> = 340  $\mu\text{l l}^{-1}$ . The sensitivity to C<sub>i</sub>, dg<sub>s</sub>/dC<sub>i</sub>, is the slope of g<sub>s</sub> versus C<sub>i</sub>, derived from the fitted quadratic function, at C<sub>i</sub> corresponding to C<sub>a</sub> = 340  $\mu\text{l l}^{-1}$ . Means of two replicates per species; n = 8.

	VPD	dg <sub>s</sub> /dC <sub>a</sub>	dg <sub>s</sub> /dC <sub>i</sub>
	kPa	mol m <sup>-2</sup> s <sup>-1</sup>	
Maize (C <sub>4</sub> )	0.50	-1,990	-2,447
	0.95	-949	-1,235
	1.41	-864	-1,093
	1.94	-698	-865
Paspalum (C <sub>4</sub> )	0.46	-2,637	-2,812
	0.93	-1,130	-1,360
	1.41	-822	-1,074
	1.94	-601	-844
Rice (C <sub>3</sub> )	0.45	-1,593	-1,878
	0.93	-628	-809
	1.41	-480	-662
	2.01	-357	-506
Phalaris (C <sub>3</sub> )	0.44	-2,447	-2,706
	0.91	-1,265	-1,498
	1.40	-594	-740
	1.99	-576	-740

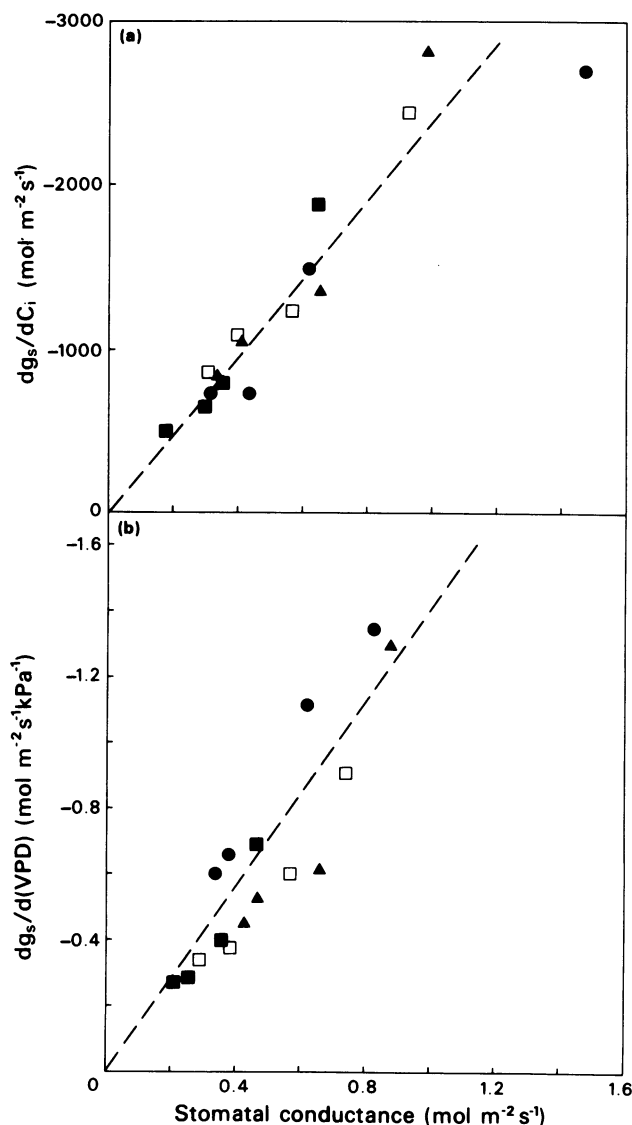


FIG. 3. Relationship between (a) stomatal sensitivity to C<sub>i</sub>, dg<sub>s</sub>/dC<sub>i</sub>, and stomatal conductance and (b) stomatal sensitivity to VPD, dg<sub>s</sub>/d(VPD), and stomatal conductance. (▲), Paspalum (C<sub>4</sub>); (□), maize (C<sub>4</sub>); (■), rice (C<sub>3</sub>); and (●), phalaris (C<sub>3</sub>). a, Dashed line is a linear regression, ( $r^2 = 0.87$ ); b, dashed line is a linear regression constrained through the origin ( $r^2 = 0.80$ ).

(but see "Discussion"). Stomatal conductance was reduced at all VPD by C<sub>a</sub> values that are higher than current atmospheric concentrations and increased at lower concentrations in all species. Leaves of phalaris (C<sub>3</sub>) and paspalum (C<sub>4</sub>) had very high stomatal conductances at small vapor pressure differences; larger VPD decreased g<sub>s</sub> at all ambient CO<sub>2</sub> concentrations in each species.

When the data are replotted as a function of C<sub>i</sub> (Figure 2), it is apparent that, at a particular C<sub>a</sub>, C<sub>i</sub> declined with increasing VPD in all four species (*i.e.* dashed line, denoting g<sub>s</sub> at C<sub>i</sub> at C<sub>a</sub> = 340  $\mu\text{l l}^{-1}$  curves to lower C<sub>i</sub>). This effect is more marked in the C<sub>4</sub> species (C<sub>i</sub> changed by up to 80  $\mu\text{l l}^{-1}$  at C<sub>a</sub> = 340  $\mu\text{l l}^{-1}$ ) than in the C<sub>3</sub> species, but had little effect on net assimilation rate in the C<sub>4</sub> species as A versus C<sub>i</sub> was close to the plateau region. Stomatal conductance showed large changes in response to both C<sub>i</sub> and VPD; there was no unique relationship between g<sub>s</sub> and C<sub>i</sub> for all vapor pressure differences.

**Analysis of Stomatal Sensitivity to CO<sub>2</sub> and VPD.** Table I shows the sensitivity of stomatal conductance to C<sub>a</sub> and to C<sub>i</sub>,

expressed as the slopes  $dg_s/dC_a$  and  $dg_s/dC_i$  at  $C_a = 340 \mu\text{l l}^{-1}$ , derived from quadratic functions fitted to  $g_s$  versus  $C_a$  and  $g_s$  versus  $C_i$  at each VPD. While the sensitivity of stomatal conductance to CO<sub>2</sub>, expressed either as  $C_a$  or as  $C_i$ , was lower with larger VPD, there were little differences between C<sub>3</sub> and C<sub>4</sub> species. Only rice showed lower mean sensitivity to both  $C_a$  and  $C_i$  than the other species ( $P < 0.05$ , analysis of variance). However, stomatal conductance was lower in rice than in the other species (Figs. 1b and 2b) suggesting that the stomatal sensitivity to CO<sub>2</sub> may have been proportional to the absolute magnitude of  $g_s$ . Figure 3a indicates that this was indeed the case; there is a single linear relationship between the stomatal sensitivity to  $C_i$  and stomatal conductance for all four species at different VPD.

Figure 4 shows the response of stomatal conductance to VPD at two ambient CO<sub>2</sub> concentrations, 340 and 790  $\mu\text{l l}^{-1}$ . The response in all four species was similar, but stomatal conductance in phalaris (C<sub>3</sub>) showed larger increases at small VPD. High ambient CO<sub>2</sub> reduced stomatal conductance at each VPD (Fig. 4) and the stomatal sensitivity to VPD. In Table II, this sensitivity is expressed as the slope  $dg_s/d(\text{VPD})$  at VPD = 0.9 kPa, derived from quadratic functions fitted to  $g_s$  versus VPD at each  $C_a$ . The sensitivity decreased with increased  $C_a$  and varied between species but was linearly related to absolute stomatal conductance in all four species and CO<sub>2</sub> concentrations (Fig. 3b).

**Transpiration and Transpiration Efficiency.** In the absence of any change in stomatal conductance, the rate of transpiration for a leaf held at constant temperature is directly proportional to VPD (e.g. 24). However, stomatal conductance responded markedly to

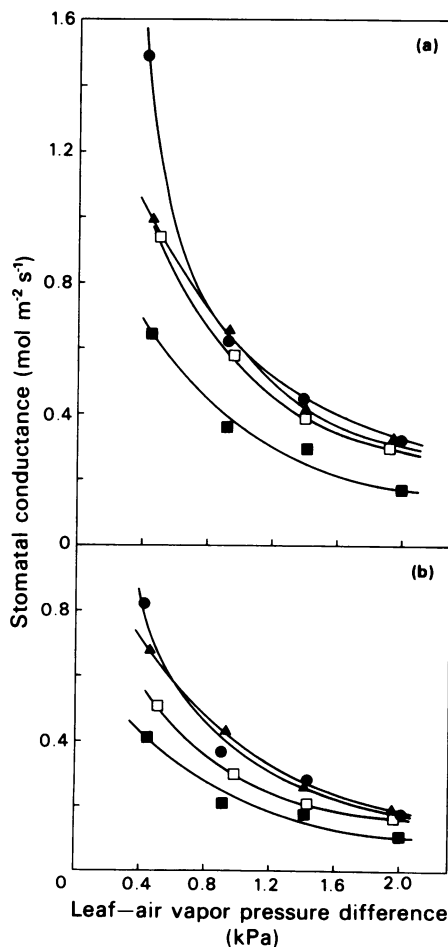


FIG. 4. Response of stomatal conductance to leaf-air vapor pressure difference at two ambient CO<sub>2</sub> concentrations (a) 340  $\mu\text{l l}^{-1}$  and (b) 800  $\mu\text{l l}^{-1}$ . Symbols as in Figure 3.

Table II. Sensitivity of Stomatal Conductance to VPD

The sensitivity to VPD,  $dg_s/d(\text{VPD})$ , is the slope of  $g_s$  versus VPD derived from the fitted quadratic function, at VPD = 0.9 kPa; means of two replicates;  $n = 8$ .

	VPD	$C_a$	$dg_s/d(\text{VPD})$
	kPa	$\mu\text{l l}^{-1}$	$\text{mol m}^{-2} \text{s}^{-1} \text{kPa}^{-1}$
Maize (C <sub>4</sub> )	0.95	190	-0.91
		340	-0.60
		580	-0.38
		790	-0.34
Paspalum (C <sub>4</sub> )	0.93	190	-1.30
		340	-0.61
		580	-0.53
		790	-0.45
Rice (C <sub>3</sub> )	0.93	190	-0.69
		340	-0.40
		580	-0.28
		790	-0.27
Phalaris (C <sub>3</sub> )	0.91	190	-1.35
		340	-1.12
		580	-0.66
		790	-0.61

VPD in the four species in this present study (Fig. 4), so much so that at VPD larger than 0.9 kPa there were only small increases in transpiration, especially at high ambient CO<sub>2</sub> concentrations (Fig. 5). In the two C<sub>3</sub> species, transpiration even declined when VPD was increased from 1.4 to 2.0 kPa, this decline coinciding with the decline in net assimilation rate (Fig. 2a). Transpiration efficiency (A/E, mmol CO<sub>2</sub> assimilation/mol H<sub>2</sub>O transpiration; Fig. 6) increased with higher atmospheric CO<sub>2</sub> in all four species, but in the two C<sub>4</sub> species was almost independent of VPD between 0.9 and 2.0 kPa because of the lack of effect of VPD on assimilation and transpiration (Figs. 1 and 5). In the two C<sub>3</sub> species, A/E decreased with larger VPD. At a vapor pressure difference and ambient CO<sub>2</sub> concentration similar to that in which the plants were grown (VPD = 0.9 kPa and  $C_a = 3 \mu\text{l l}^{-1}$ , arrowed points), C<sub>4</sub> plants had a higher transpiration efficiency than the C<sub>3</sub> plants (7.1 for maize [C<sub>4</sub>], 5.0 for paspalum [C<sub>4</sub>], 4.5 for rice [C<sub>3</sub>], and 3.1 mmol mol<sup>-1</sup> for phalaris [C<sub>3</sub>]) as expected. However, the relative increase of A/E with increased ambient CO<sub>2</sub> from 340 to 800  $\mu\text{l l}^{-1}$  was larger for the C<sub>3</sub> species than for the C<sub>4</sub> species;  $[(A/E \text{ at } C_a = 800) - (A/E \text{ at } C_a = 340)] / (A/E \text{ at } C_a = 340)$  was 0.7 in maize, 0.4 in paspalum, 1.2 in rice, and 1.0 in phalaris (average of four VPD).

**The Relationship between  $C_i$  and  $C_a$ .** Figure 7 shows the relationship between  $C_i$  and  $C_a$  while VPD was maintained constant at 0.9 kPa—a humidity close to that at which the plants were grown. The relationships are all close to linear but the extrapolated regressions of the C<sub>4</sub> species intersect the abscissa at  $C_a \approx 50 \mu\text{l l}^{-1}$  and not near the origin. We conclude that the relationship was not truly linear for maize and paspalum, with  $C_i/C_a$  increasing slightly as  $C_a$  increases. However, there is no clear distinction between the values of  $C_i/C_a$  for C<sub>3</sub> and C<sub>4</sub> species. At  $C_a = 340 \mu\text{l l}^{-1}$ , the value of  $C_i/C_a$  was largest in phalaris (C<sub>3</sub>; 0.85) and lowest in maize (C<sub>4</sub>; 0.67), but identical in paspalum (C<sub>4</sub>) and rice (C<sub>3</sub>; 0.78).

However,  $C_i/C_a$  decreased with larger VPD (Fig. 8) in each species as a result of the reduced stomatal conductance. Only at large VPD (2.0 kPa) in rice and phalaris was net assimilation affected (Fig. 1) causing a higher  $C_i/C_a$  than predicted by the linear relationship between  $C_i/C_a$  and VPD. The slope of the relationship between  $C_i/C_a$  and VPD was largest in the C<sub>4</sub> species,

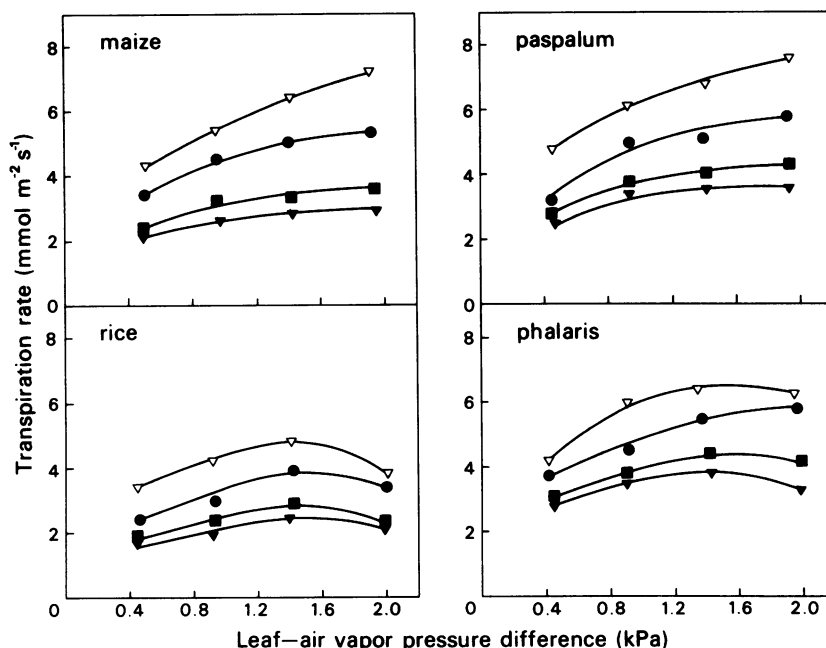


FIG. 5. Response of transpiration rate to leaf-air vapor pressure difference at four ambient  $\text{CO}_2$  concentrations: ( $\nabla$ ),  $200 \mu\text{l l}^{-1}$ ; ( $\bullet$ ),  $340 \mu\text{l l}^{-1}$ ; ( $\blacksquare$ ),  $600 \mu\text{l l}^{-1}$ ; ( $\blacktriangledown$ ),  $800 \mu\text{l l}^{-1}$ .

particularly maize, so that at 2.0 kPa  $C_i/C_a$  had declined to 0.45 (Fig. 8) in this species.

## DISCUSSION

The results do not support the assumption that the stomata of  $C_4$  species are always more sensitive to  $\text{CO}_2$  than those of  $C_3$  species, whether the sensitivity is expressed with respect to ambient  $\text{CO}_2$  or to intercellular  $\text{CO}_2$  concentration. It might be argued that in these well-watered plants the levels of ABA were low, reducing the sensitivity of stomata to  $\text{CO}_2$  (e.g. 22, 23). However, we measured high sensitivity to  $\text{CO}_2$ , and the values of  $dg_s/dC_i$  (Table I) were at the upper end of the range reported by other workers (3, 5, 23). Furthermore, the values of stomatal conductance under conditions similar to those in which the plants were grown were large and similar in the  $C_4$  and  $C_3$  species, contrary to many reports that stomatal conductance is larger in  $C_3$  plants than  $C_4$  (2, 7). However, a recent review of many measurements of maximum stomatal conductance concluded that there is no indication of a generally lower maximum conductance in  $C_4$  grasses as compared with  $C_3$  grasses (13). The average values of maximum conductance for grasses was about  $0.45 \text{ mol m}^{-2} \text{ s}^{-1}$  (Ref. 13; Fig. 2), this being lower than the values observed in this study at 0.9 kPa VPD and  $C_a = 340 \mu\text{l l}^{-1}$  (Fig. 1b).

Farquhar and Sharkey (4) have recently argued that stomata usually impose only a slight limitation on net assimilation, and further, that stomata do not limit assimilation in  $C_4$  species more than in  $C_3$  species. Figure 2a supports this argument as the net assimilation rates at  $C_a = 340 \mu\text{l l}^{-1}$  in the  $C_4$  species occur, over a range of VPD, near the saturated region of the response curve of assimilation to  $C_i$  where there is little or no stomatal limitation. In  $C_3$  species, there was only a small limitation shown in Figure 2a as the difference in assimilation rate in the region bounded by arrows to that at \*, the point where stomatal conductance imposes no limitation and leaf conductance equals the boundary layer conductance, following Farquhar and Sharkey (4).

Stomatal conductance was sensitive to humidity in all four species, and low  $\text{CO}_2$  increased the response to VPD and vice versa (Figs. 1b and 2b); however, the relative response of  $g_s$  to either  $\text{CO}_2$  or VPD was unaffected by the other variable: the

sensitivity of stomata to  $\text{CO}_2$  or VPD was proportional to the absolute  $g_s$  (Fig. 3, a and b). Similar results in *Sesamum indicum* (9) and *Vigna unguiculata* (10) support this conclusion. The similarity of the stomatal sensitivities to  $\text{CO}_2$  and to VPD among the four species examined is surprising in view of the different responses of stomata to environmental variables observed previously between closely related species and even clones (20). The linear relationship between the sensitivity of stomatal conductance to  $\text{CO}_2$  and absolute stomatal conductance as VPD decreased (Fig. 3a) contrasts with the effects of ABA and light on stomatal sensitivity to  $\text{CO}_2$  and stomatal conductance. Increased ABA supply reduces conductance but increases the sensitivity of stomata to  $\text{CO}_2$  (e.g. 3, 22, 23) and increasing light intensity increases conductance but reduces stomatal sensitivity to  $\text{CO}_2$  at low  $\text{CO}_2$  concentrations (17, 25). This interaction of light, ABA, and  $\text{CO}_2$  on stomata has previously suggested that these factors work at a common level in the stomatal mechanism (16, 17). The observation that VPD does not affect the  $\text{CO}_2$  response of stomata in the same direction as does ABA or light emphasizes therefore that VPD acts at a different level.

In contrast to the stomatal response to humidity, assimilation by the mesophyll was largely unaffected by humidity. Presuming that mesophyll assimilation rate is sensitive to leaf water potential changes, the lack of any effect of VPD on net assimilation rate indicates a 'direct' effect of VPD on stomata, i.e. not mediated by changes in bulk  $\Psi$ . The only exception to this, the decline of assimilation rate and transpiration rate at large VPD of 2.0 kPa in rice and phalaris (Figs. 2 and 5) is, however, difficult to understand. Assimilation rate is affected even at saturating  $C_i$  (Fig. 2), yet  $\Psi$  cannot be less than at 1.4 kPa VPD as the rate of transpiration is actually lower. It is possible that the change to 2.0 kPa VPD caused a transient decline in  $\Psi$  which reduced net assimilation rate and conductance. However, recovery from any such transient condition was not observed over the 5 h required for measuring the  $\text{CO}_2$  response curve at 2.0 kPa VPD; conditions appeared to be steady. We can only suggest some localized drying effect on the mesophyll tissue.

The sensitivity of stomata to VPD caused changes in the ratio  $C_i/C_a$ . In all four species,  $C_i/C_a$  was nearly independent of  $C_a$ , but was linearly related to VPD (Figs. 7 and 8). At the extreme of

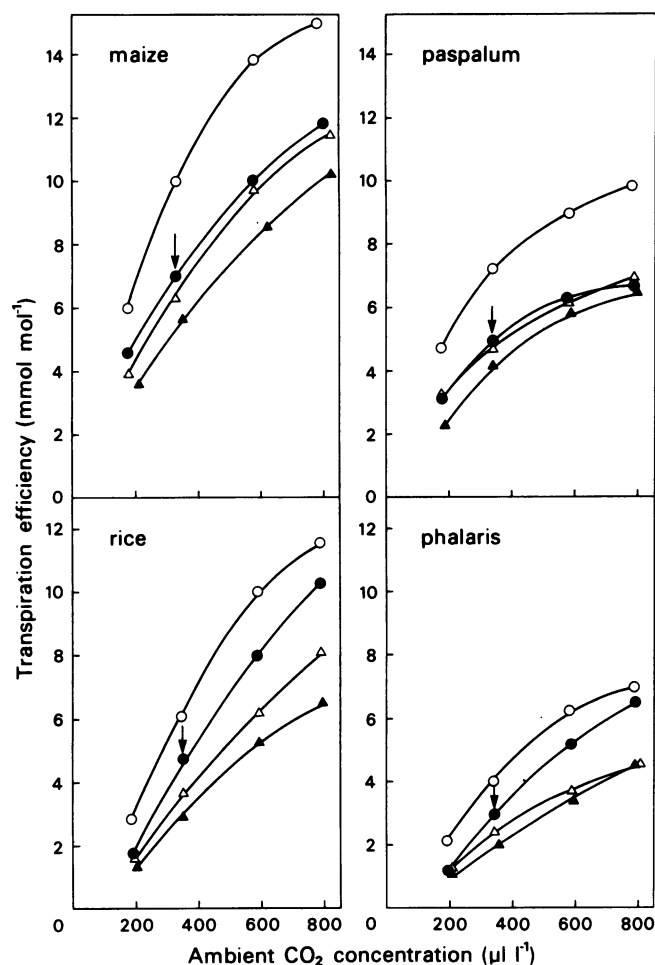


FIG. 6. Relationship between transpiration efficiency ( $\text{mmol [CO}_2\text{] m}^{-2} \text{s}^{-1} / \text{mol [H}_2\text{O] m}^{-2} \text{s}^{-1}$ ) and  $C_a$  at four VPD levels; same symbols as Figure 1. Arrows indicate transpiration efficiencies at VPD = 0.9 kPa,  $C_a = 340 \mu\text{l l}^{-1}$ .

water-saturated air,  $C_i/C_a$  (by extrapolation) would be 0.8 to 0.9 for all species, C<sub>3</sub> or C<sub>4</sub>. Only at the largest vapor pressure difference was  $C_i/C_a$  substantially different between the two C<sub>3</sub> and C<sub>4</sub> species, and comparable with 'typical' measurements in the literature. VPD may have a large effect on  $C_i/C_a$  in other species. For example, in the C<sub>4</sub> dicot, *Amaranthus powelli*  $C_i/C_a$ , measured at 0.7 kPa VPD, was 0.7 (3), similar to  $C_i/C_a$  in maize in the present study at the same VPD. However, the importance of the VPD effect on  $C_i/C_a$  in the field cannot be estimated, as the response of stomata to both humidity and CO<sub>2</sub> varies with growing conditions (e.g. 3, 5, 20, 23).

Many workers have reported that C<sub>4</sub> plants have higher transpiration efficiencies (units CO<sub>2</sub> gain/units H<sub>2</sub>O loss) than C<sub>3</sub> plants (e.g. 24). At  $C_a = 340 \mu\text{l l}^{-1}$  and VPD = 0.9 kPa, our results agree with such reports (Fig. 6). However, because the relative increase of A/E with increased  $C_a$  was larger in the C<sub>3</sub> plants than in the C<sub>4</sub> plants, the transpiration efficiency of the two C<sub>3</sub> species at  $C_a = 800 \mu\text{l l}^{-1}$  exceeded that of the C<sub>4</sub> species paspalum. It remains to be seen whether such increases of transpiration efficiency of individual leaves are matched by similar increases in the water use efficiency of crops as the atmospheric CO<sub>2</sub> concentration increases.

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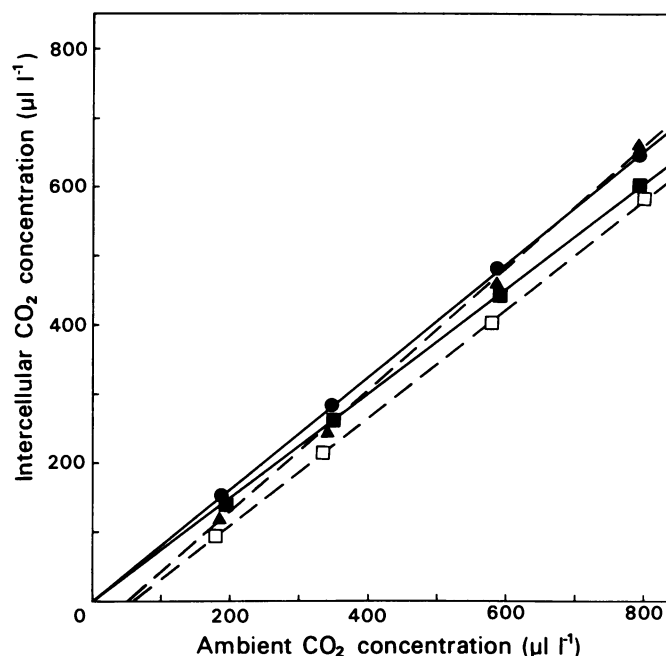


FIG. 7. The relationship between intercellular CO<sub>2</sub> concentration and ambient CO<sub>2</sub> concentration at constant VPD (0.9 kPa). Symbols as in Figure 3. Linear regressions extrapolated to x axes ( $r^2 = 1.00$ ).

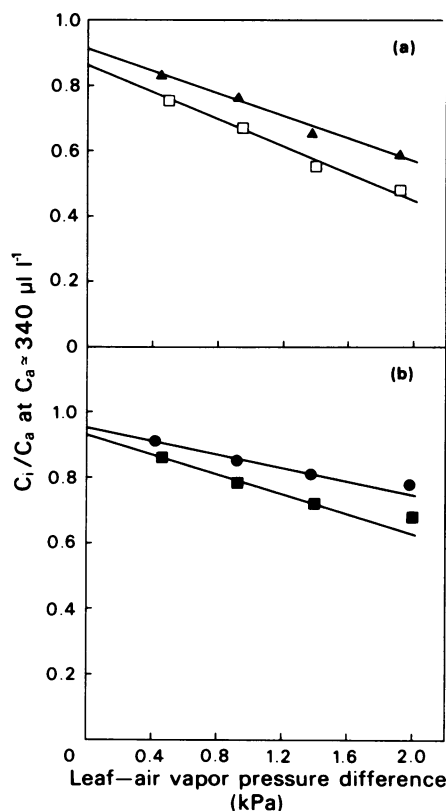


FIG. 8. The relationship between  $C_i/C_a$  at  $C_a = 340 \mu\text{l l}^{-1}$  and leaf-air vapor pressure difference for (a) maize and paspalum (C<sub>4</sub>) and (b) rice and phalaris (C<sub>3</sub>). Symbols as in Figure 3. Linear regressions extrapolated to y axes ( $r^2 = 0.90-0.97$ ).

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